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Attorney Docket N . UMY-088-1
Customer No. 00959

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Fumihiko Urano

For: Methods and Reagents to Quantify Endoplasmic Reticulum Stress (ER Stress)

Mail Stop Provisional Patent Application
Commissioner for Patents
Post Office Box 1450
Alexandria, VA 22313-1450

00746 U.S. PTO
60/510262



100903

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: October 9, 2003

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I hereby certify that this Cover Sheet for Filing Provisional Application (37 C.F.R. §1.51(2)(i)) and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Mail Stop Provisional Patent Application, Commissioner for Patents, Post Office Box 1450, Alexandria, VA 22313-1450.

Debra J. Milasincic

Name of Person Mailing Paper

Signature of Person Mailing Paper

COVER SHEET FOR FILING U.S. PROVISIONAL PATENT APPLICATION

Dear Sir:

The accompanying application, entitled "*Methods and Reagents to Quantify Endoplasmic Reticulum Stress (ER Stress)*", is a provisional patent application under 37 C.F.R. §1.51(a)(2) and §1.53(b)(2).

1. ☒ The name and address of the inventor of this application is as follows:

Last Name	First Name	Middle Initial	Residence
Urano	Fumihiko		58 Elgin Street Newton, MA 02459

2. ☒ The following documents are enclosed:

- ☒ 12 pages of Specification;
- ☒ 2 pages of Claims;
- ☒ 1 page of Abstract;
- ☒ 4 sheets of Drawings (*Figures 1-4*); and
- ☒ an acknowledgment postcard.

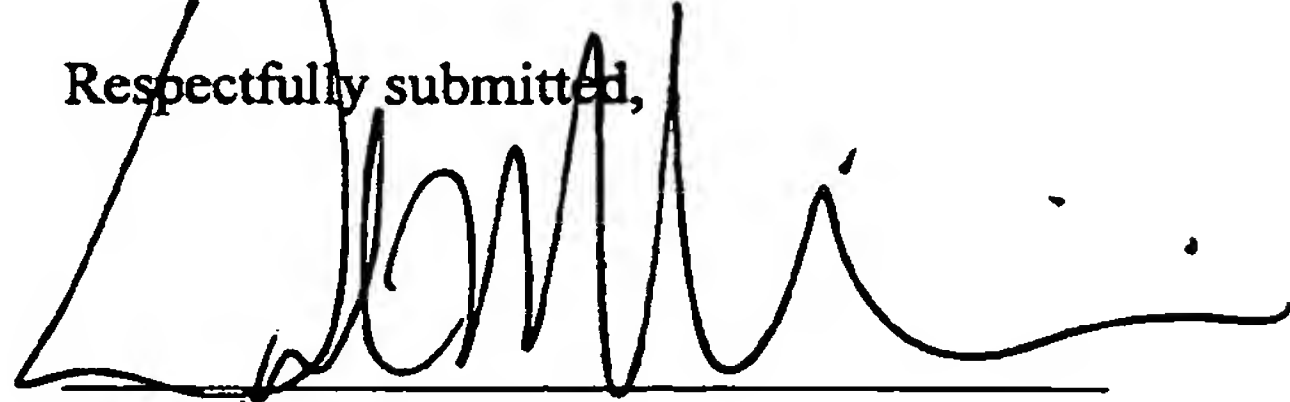
3. ☒ Applicant claims small entity status under 37 C.F.R. §1.27.
4. ☒ The fee for filing this provisional application, as set forth in 37 CFR 1.16(k), is \$80.00.
- a. ☐ A check for this filing fee is enclosed.
- b. ☒ The Commissioner is authorized to charge the filing fee to our Deposit Account No. 12-0080.
- c. ☐ The filing fee is not being paid at this time.
5. ☒ Please charge any fee deficiencies associated with this filing to Deposit Account No. 12-0080. *A duplicate copy of this sheet is enclosed.*

6. ☒ Please address all future communications to **Debra J. Milasinsic, Esq.** at **Customer Number 000959** whose address is:

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METHODS AND REAGENTS TO QUANTIFY ENDOPLASMIC RETICULUM STRESS (ER STRESS)

Government Rights

5 This invention was made at least in part with government support under grant no. R01 DK067493-01 awarded by the National Institutes of Health. The government may have certain rights in this invention.

Background of the Invention

10 Proteins are needed for the body to function properly. They are the basis of body structures and are used to synthesize enzymes and antibodies in cells. Proteins must fold into a proper three-dimensional structure to carry out their functions. However, sometimes the protein folding goes awry and misfolded proteins accumulate in cells, causing or contributing to protein conformational diseases, for example, Alzheimer's
15 disease and cystic fibrosis.

 Proteins destined for secretion such as insulin are translocated into the endoplasmic reticulum (ER) co-translationally and undergo highly ordered protein folding and post-translational protein processing. However, in some instances, the sensitive folding environment in the ER can be perturbed by pathophysiological
20 processes such as viral infections, environmental toxins, and mutant protein expression, as well as natural processes such as the large biosynthetic load placed on the ER. When the demand that the load of client proteins makes on the ER exceeds the actual folding capacity of the ER to meet that demand, a situation termed "ER stress" results. There exists a need for methods of quantifying ER stress.

25

Summary of the Invention

 To date there has not been described a way to quantify the level of ER stress. The instant invention provides novel methods and reagents for quantifying ER stress. In particular, the methods feature IRE1 and XBP-1 as specific markers for ER stress level.
30 As a marker, XBP-1 offers an advantage because we can quantify the ER stress level from a small biological sample by monitoring the splicing of XBP-1 mRNA. Exemplary methods are based on PCR. Thus, one only needs a small tissue sample or a small amount of cells for this assay.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

5

Brief Description of the Drawings

Figure 1. IRE1-mediated processing of XBP-1 mRNA during ER stress. (A) Map of the unspliced and spliced mouse XBP-1 mRNAs. The coding regions are boxed, the bZip domain is shaded grey and the 26-bp nucleotide region processed by IRE1 is colored black. The active form of XBP-1 mRNA (cDNA) loses 26 base pairs by IRE1 processing. Spliced form of XBP-1 mRNA encodes larger and active form of XBP-1 protein. The inactive form of XBP-1 cDNA is smaller than the DNA fragment of active form of XBP-1. (B) Wild-type or IRE1 mutant mouse embryonic fibroblast cells were untreated or treated with Tunicamycin (TM) or Thapsigargin (Tg). Total RNAs were prepared at the indicated times. RT-PCR analysis was done with a primer set encompassing the splice junction of XBP-1 mRNA. PCR products were resolved on 2.5 % agarose gel to separate spliced (active form) and unspliced XBP-1 mRNAs.

Figure 2. IRE1-mediated processing of XBP-1 mRNA during ER stress. (A) Map of the unspliced and spliced murine XBP-1 mRNAs. The coding regions are boxed, the bZip domain is shaded grey and the 26-bp nucleotide region processed by IRE1 is colored black. The active form of XBP-1 mRNA (cDNA) loses its Pst I site by IRE1 processing. Spliced form of XBP-1 mRNA encodes larger and active form of XBP-1 protein. The inactive form of XBP-1 cDNA digested by Pst I shows two DNA fragments that are smaller than the DNA fragment of active form of XBP-1 digested by Pst I. (B) Pst I digested XBP-1 cDNA from wild-type or IRE1 mutant cells that were untreated or treated with Tunicamycin (TM) or Thapsigargin (Tg).

Figure 3. Processing of XBP-1 mRNA in islet cells. Pst I digested Xbp-1 cDNA from mouse islet cells that were untreated (Control) or treated with 1mM of dithiothreitol (DTT) for 4 hours. The spliced (encoding active form of XBP-1) and unspliced (encoding inactive form of XBP-1) cDNA fragments are indicated by the arrows.

Figure 4. XBP-1 splicing in MIN-6 cells expressing insulin-2 gene with Akita mutation. Pst I digested XBP-1 cDNA from MIN6 cells untransfected, transfected with

wild-type Insulin 2 expression vector or with insulin-2 containing Akita mutation expression vector.

Detailed Description of the Invention

5 Since the ER stress signaling network plays a role in the pathogenesis of human diseases, it is important to monitor the ER stress level in mammalian cells. The present invention provides methods and reagents to quantify ER stress level.

 IRE1 is one of the most upstream components of ER stress signaling network and it is a sensor for ER stress. The present invention features quantifying IRE1
10 activation levels as a measure of ER stress. Because it is difficult to measure the IRE1 activation level by itself, XBP-1 mRNA splicing level, which reflects the IRE1 activation level, is used to quantify the IRE1 activation level. Spliced XBP-1 mRNA encodes the active transcription factor and activates the UPR. The invention features a method to quantify the activation level of XBP-1 by Reverse Transcriptase-PCR (RT-
15 PCR). Primers are designed to amplify the region encompassing the splice junction of XBP-1 mRNA. The spliced form (the active form) of XBP-1 mRNA (cDNA) is smaller than the unspliced form (inactive form) by 26bp. The size difference between the two forms can be visualized, for example, by electrophoresing the PCR products on agarose gel.

20

 So that the invention may be more readily understood, certain terms are first defined.

 The term “endoplasmic reticulum stress” (“ER stress”) refers to the imbalance
25 between the demand that the load of client proteins makes on the ER and the actual folding capacity of the ER to meet that demand. The response that counteracts ER stress has been termed “unfolded protein response” (“UPR”).

 The term “ER stress disorder” refers to a disease or disorder (*e.g.*, a human disease or disorder) caused by or contributed to by increased ER stress levels. An
30 exemplary ER stress disorder is diabetes (*e.g.*, juvenile diabetes).

 The term “protein conformational disease” refers to a disease or disorder (*e.g.*, a human disease or disorder) associated with protein misfolding (*e.g.*, caused by or contributed to by protein misfolding). Exemplary protein conformational diseases

include, but are not limited to, Alzheimer's disease (caused by the aggregation of amyloid fibrils) and cystic fibrosis (caused by the malfunctioning of an ion channel protein).

The term "RNA" or "RNA molecule" or "ribonucleic acid molecule" refers to a polymer of ribonucleotides. The term "DNA" or "DNA molecule" or deoxyribonucleic acid molecule" refers to a polymer of deoxyribonucleotides. DNA and RNA can be synthesized naturally (*e.g.*, by DNA replication or transcription of DNA, respectively). RNA can be post-transcriptionally modified. DNA and RNA can also be chemically synthesized. DNA and RNA can be single-stranded (*i.e.*, ssRNA and ssDNA, respectively) or multi-stranded (*e.g.*, double stranded, *i.e.*, dsRNA and dsDNA, respectively). "snRNA" or "small nuclear RNA" is single-stranded RNA precursor of mRNA. "mRNA" or "messenger RNA" is single-stranded RNA that specifies the amino acid sequence of one or more polypeptide chains. This information is translated during protein synthesis when ribosomes bind to the mRNA.

Various aspects of the invention are described in further detail in the following subsections.

I. ER stress and ER stress signaling pathways

The unfolded protein response (UPR) is a cellular adaptive response that counteracts the ER stress. The UPR has three different arms to deal with the ER stress: (1) gene expression, (2) translational attenuation, and (3) protein degradation. Recently, we have demonstrated that Inositol Requiring 1 (IRE1), an ER-resident transmembrane protein kinase, is one of the upstream components of the UPR and a central regulator for UPR-specific downstream gene expression and apoptosis.

IRE1 is a sensor of unfolded proteins in the ER. IRE1 is a unique upstream component of UPR because it can serve both as pro-apoptotic and anti-apoptotic factors. The presence of unfolded proteins in the ER causes dimerization and trans-autophosphorylation of IRE1 that lead to the IRE1 activation. We have shown that activated IRE1 splices X-Box Binding Protein-1 (XBP-1) mRNA that leads to synthesis of the active transcription factor XBP-1 and upregulation of UPR genes. In contrast, prolonged ER stress activates the cell death pathway through IRE1.

IRE1 and XBP-1 are crucial components of unfolded protein response, and the expression levels of the active forms of XBP-1 and IRE1 can serve as markers for ER

stress levels. It is difficult to measure the activation level of IRE1. Activation of IRE1 by its phosphorylation causes its shift to lower mobility on SDS-polyacrylamide gel. However, the shift by its activation is very small and difficult to detect. We use XBP-1 as our measure of ER stress level because we have developed a method to quantify the activation level of XBP-1 by RT-PCR, optionally followed by Pst-I digestion (See Examples 2-4). The mRNA sequences for the spliced and unspliced forms of XBP-1 are as follows.

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        Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
    REFERENCE  1
20  AUTHORS    Yoshida,H., Matsui,T., Yamamoto,A., Okada,T. and Mori,K.
    TITLE      XBP1 mRNA Is Induced by ATF6 and Spliced by IRE1 in Response to ER
        Stress to Produce a Highly Active Transcription Factor
        JOURNAL Cell (2001) In press
    REFERENCE  2 (bases 1 to 1761)
25  AUTHORS    Yoshida,H. and Mori,K.
    TITLE      Direct Submission
    JOURNAL    Submitted (18-DEC-2001) Hiderou Yoshida, Kyoto University, Graduate
        School of Biostudies; 46-29 Yoshida-Shimoadachi-machi, Sakyo-ku,
        Kyoto, Kyoto 606-8304, Japan
30  (E-mail:hidyoshi@ip.media.kyoto-u.ac.jp,
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30 LOCUS AB076383 1787 bp mRNA linear PRI 05-JAN-2002
DEFINITION Homo sapiens XBP1 mRNA for X box-binding protein unspliced form,
complete cds.
ACCESSION AB076383
VERSION AB076383.1 GI:18148379
35 KEYWORDS .
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ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
40 REFERENCE 1
AUTHORS Yoshida,H., Matsui,T., Yamamoto,A., Okada,T. and Mori,K.
TITLE XBP1 mRNA Is Induced by ATF6 and Spliced by IRE1 in Response to ER
Stress to Produce a Highly Active Transcription Factor
JOURNAL Cell (2001) In press
45 REFERENCE 2 (bases 1 to 1787)
AUTHORS Yoshida,H. and Mori,K.
TITLE Direct Submission
JOURNAL Submitted (18-DEC-2001) Hiderou Yoshida, Kyoto University, Graduate
School of Biostudies; 46-29 Yoshida-Shimoadachi-machi, Sakyo-ku,
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40      //

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Primers for amplifying a region of the human XBP-1 mRNA that includes a splice junction correspond (or are reverse complements) of the underlined sequences. Additional primer pairs can readily be designed by the skilled artisan given the above sequences and primer design programs.

45

II. Uses

Quantifying or detecting ER stress is useful in any situation where it is suspected or has been determined that such stress may regulate a normal cellular phenotype (*e.g.*, regulate apoptosis) or cause or contribute to a disease phenotype (*e.g.*, a protein conformational disease phenotype). In mammalian cells, ER stress is regulated, at least in part, by an ER stress signaling pathway. This pathway is an evolutionarily conserved signaling network that is emerging as the major quality controller of newly synthesized proteins. Preliminary data suggest that ER stress signaling is crucial for protein

secretion and the development of secretory cells, such as plasma cells, adipocyte and trophoblast cells in placenta. These data also suggest that defects in this signaling network can cause or contribute to human diseases, such as Alzheimer's disease, inflammatory Bowel disease, diabetes mellitus, and cancers originated from secretory
5 cells (breast cancer, prostate cancer). The methods and reagents of the invention are suitable for use in research methods to further study the role of ER stress in cellular processes such as apoptosis and contribution of such processes in a variety of ER stress diseases.

The methods of the invention are also suitable for use in methods of diagnosing
10 ER stress diseases. The methods and reagents are particularly suitable for diagnosing diabetes, in particular, juvenile diabetes, as this disease is believed to be caused, at least in part by increases ER stress. More than one million people suffer from juvenile diabetes (type 1) in the U.S. In this disease, insulin production is abnormally low because beta-cells in pancreatic islets are destroyed. Recent observations in the Akita
15 diabetes model mouse in combination with findings by the instant inventor support the hypothesis that sufficient endoplasmic reticulum (ER) stress can cause beta-cell death.

It is believed that defects in the ER stress signaling network also causes or contributes to human diseases such as Alzheimer's disease, inflammatory bowel disease and cancers originating from secretory cells (*e.g.*, breast cancer and prostate cancer).
20 Thus it is contemplated that the ER stress quantification methodologies of the instant invention may also be useful in methods for diagnosing any of this disease in patients. In an exemplary embodiment, the methods and reagents described herein can be used to diagnose the stage of multiple myeloma in patients.

Various methodologies of the instant invention include step that involves
25 comparing a value, level, feature, characteristic, property, etc. to a "suitable control", referred to interchangeably herein as an "appropriate control". A "suitable control" or "appropriate control" is any control or standard familiar to one of ordinary skill in the art useful for comparison purposes. In one embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined
30 prior to performing a methodology of the invention described herein. In another embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined in a cell or organism, *e.g.*, a control or normal cell or organism, exhibiting, for example, normal traits. In yet another embodiment, a

“suitable control” or “appropriate control” is a predefined value, level, feature, characteristic, property, etc.

5 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

10

Example 1: XBP-1 splicing assay

RNA from cells is reverse transcribed using Oligo-dT primer. PCR is performed using following primers:

the sense primer hXBP1.1S: AAACAGAGTAGCAGCTCAGACTGC; and the
15 antisense primer hXBP1.2AS: TGGGCAGTGGCTGGATGAAAGC (for human),
the sense primer mXBP1.3S: AAACAGAGTAGCAGCGCAGACTGC and the
antisense primer mXBP1.6AS: CAGACAATGGCTGGATGAAAGC (for mouse), and
the sense primer rXBP1.3S: AAACAGAGTAGCAGCACAGACTGC and the
anti-sense primer mXBP1.6AS: CAGACAATGGCTGGATGAAAGC (for rat).

20 PCRs amplify a 768-bp cDNA product for human, a 774-bp cDNA product for mouse, and a 774-bp cDNA product for rat (unspliced forms). These cDNAs encompass the IRE1 cleavage sites.

The thermal cycle reaction is performed as follows: 94°C for 3 minutes,
followed by 35 cycles at 94°C for 1 minute, 62°C for 1 minute, 72°C for 1 minute, and
25 72°C for 10 minutes.

These fragments can be further digested by Pst-I to reveal restriction sites that are lost after IRE1-mediated cleavages and splicing reactions of the mRNA. The unspliced forms of XBP-1 yield 768-bp for human, 774-bp for mouse and rat. The spliced forms of XBP-1 yield 285 bp and 483 bp fragments for human, 291 bp and 483
30 bp fragments for mouse and rat.

Example 2:

The invention relates to a method to quantify the activation level of XBP-1 by Reverse Transcriptase-PCR (RT-PCR). Primers were designed to amplify the region encompassing the splice junction of XBP-1 mRNA. The spliced form (the active form) of XBP-1 mRNA (cDNA) is smaller than the unspliced form (inactive form) by 26bp. The size difference between two forms could be visualized by running the PCR product on 2.5% agarose gel (Figure 1A). To validate the method, RT-PCR analysis was carried out using mRNA from wild-type mouse fibroblast cell line and Ire1 α : Ire1 β double knock-out cell line. Those cell lines were treated with tunicamycin or thapsigargin for indicated period of time. Tunicamycin causes ER stress experimentally by blocking N-linked glycosylation which is a crucial step for protein folding in the ER. Thapsigargin also induces ER stress experimentally by altering Calcium ion concentration in the ER. RT-PCR analysis detected predominantly smaller fragment corresponding to active form (spliced form) of XBP-1 mRNA in wild-type cell line treated with tunicamycin or thapsigargin. In contrast, the same analysis detected only inactive form of XBP-1 mRNA in Ire1 α ^{-/-}: Ire1 β ^{-/-} double knock-out cell line (Figure 1B).

Example 3:

The results of the experiment described above can also be quantitated using an intermediate step of Pst I cleavage to facilitate distinguishing between spliced and unspliced XBP-1. In this Example, RT-PCR was followed by Pst I digestion. Primers were designed to amplify the region encompassing the splice junction of XBP-1 mRNA. Since the splice junction contains Pst-I site, the spliced form (the active form) of XBP-1 mRNA (cDNA) loses its Pst-I site by IRE1 processing. Pst-I digestion of RT-PCR product detects non-digested larger fragment corresponding to active form (spliced form) of XBP-1 mRNA and digested fragments (two smaller fragments) corresponding to inactive form (unspliced form) (Figure 2A). Pst-I digestion of RT-PCR product generated as described above detected predominant non-digested fragment corresponding to active form (spliced form) of XBP-1 mRNA in wild-type cell line treated with tunicamycin or thapsigargin. In contrast, the same analysis detected only inactive form of XBP-1 mRNA in Ire1 α ^{-/-}: Ire1 β ^{-/-} double knock-out cell line (Figure 2B).

Example 4:

To determine whether ER stress signaling is activated in islet cells under physiological conditions, we monitored the XBP-1 splicing in freshly isolated islet cells (Figure 3). High level of XBP-1 mRNA splicing was detected in the islet cells. DTT treatment enhanced the XBP1 splicing. It is known that DTT blocks the disulfide bond formation experimentally. This caused the ER stress and activated XBP-1 splicing.

Example 5: Insulin-2 mutation in Akita mouse causes ER stress in MIN6 cells

The Akita diabetes model mouse is a C57BL/6 mouse which is heterozygous for a mutation in insulin 2 gene. This mutation results in an amino acid substitution, cysteine 96 to tyrosine. Cysteine 96 is involved in the formation of one of the two disulfide bonds between the A and B chains of mature insulin. It is likely that this mutation causes incorrect folding of insulin precursor in the endoplasmic reticulum (ER) of pancreatic beta-cells. Diabetes in the Akita mouse is accompanied by neither obesity nor insulinitis. These mice spontaneously develop diabetes with dramatic reduction in beta-cell mass. Symptoms include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 4 weeks of age. This condition in Akita mouse is termed “diabetes”.

Since the phenotype of Akita mouse is caused by a mutation which can cause conformational changes in the insulin 2 (Ins2) gene product (81), it is hypothesized that pancreatic cells in Akita mice are under ER stress, and this stress can cause beta cell death. To test this hypothesis, we measured the XBP-1 splicing level in mouse insulinoma cells (MIN6 cells) expressing insulin-2 gene with the Akita mutation versus cells expressing wild-type insulin-2 gene. MIN6 were cultured in 10cm collagen-coated dishes in DMEM supplemented with 25mM glucose and 15% FCS. Plasmid DNAs were transfected into cell lines using Fugene (Roche, Basel, Switzerland). High XBP-1 splicing level which reflected high ER stress level was detected in the MIN-6 cells expressing mutant insulin 2 gene (Figure 4).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.

What is claimed:

1. A method of quantifying endoplasmic reticulum stress (ER stress), the method comprising:
 - 5 (a) detecting an IRE1 activation level in a cell or biological sample, wherein the IRE1 activation level correlates with ER stress, and
 - (b) quantifying said IRE1 activation level,such that ER stress is quantified.
- 10 2. The method of claim 1, wherein the IRE1 activation level is determined by detecting an XBP-1 splicing level.
3. The method of claim 2, wherein the XBP-1 splicing level is determined by:
 - 15 (a) amplifying a XBP-1 mRNA region which includes a splice site, or portion thereof;
 - (b) detecting the size of the amplified mRNA, wherein the size is indicative of spliced or unspliced mRNA;such that the XBP-1 splicing level is determined.
- 20 4. The method of claim 3, wherein the amplified mRNA is subjected to restriction enzyme digestion to facilitate detection of spliced or unspliced mRNA.
5. The method of claim 4, wherein the restriction enzyme digestion is Pst I
25 digestion.
6. The method of any one of the preceding claims, wherein the ER stress level is quantified in a cell.
- 30 7. The method of any one of the preceding claims, wherein the ER stress level is quantified in a mammalian cell.

8. The method of any one of the preceding claims, wherein the ER stress level is quantified in a human cell.

9. The method of claim 8, wherein the cell is a pancreatic beta cell.

5

10. The method of any one of claims 1-5, wherein the ER stress level is quantified in a cell extract.

10 11. A method of diagnosing an ER stress disease in a subject, comprising quantifying the level of ER stress in a cell or biological sample isolated from the subject according to the method of any one of the preceding claims, wherein an increased level of ER stress is indicative of the ER stress disease.

15 12. A method of monitoring the progression of a ER stress disease in a subject, comprising quantifying the level of ER stress in a cell or biological sample isolated from the subject at sequential time points according to the method of any one of the preceding claims, wherein an changes in level of ER stress are indicative of the progress of the ER stress disease.

20 13. The method of claim 11 or 12, wherein the ER stress disease is diabetes.

14. An ER stress signaling pathway assay comprising the step of determining the level of ER stress according to the method of any one of the preceding claims.

25 15. A kit for quantifying ER stress, comprising:
(a) primers for amplifying a region of XBP-1 mRNA which includes a splice site, or portion thereof, and
(b) instructions for use.

30 16. The kit of claim 15, further comprising a suitable control.

**METHODS AND REAGENTS TO QUANTIFY
ENDOPLASMIC RETICULUM STRESS (ER STRESS)**

Abstract of the Disclosure

- 5 The present invention provides methods and reagents to quantify endoplasmic reticulum stress (ER stress) levels. Methods for quantifying ER stress in mammalian cells are exemplified.

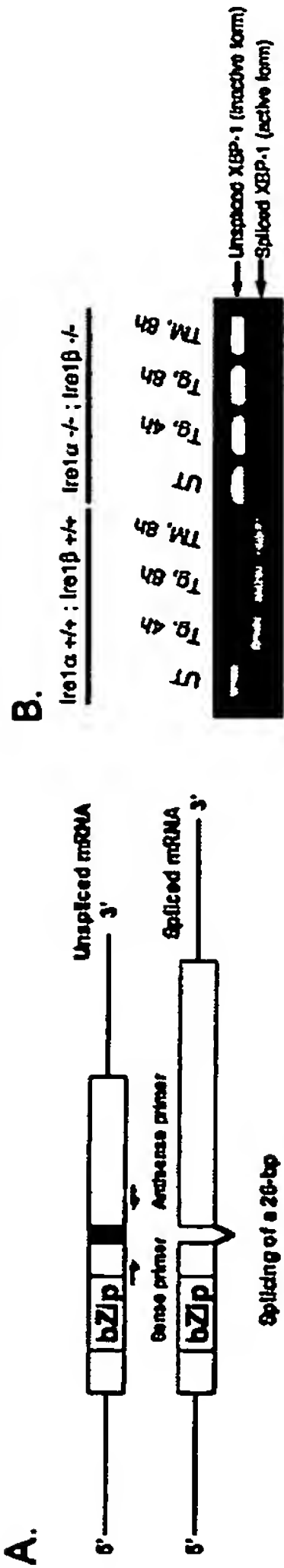
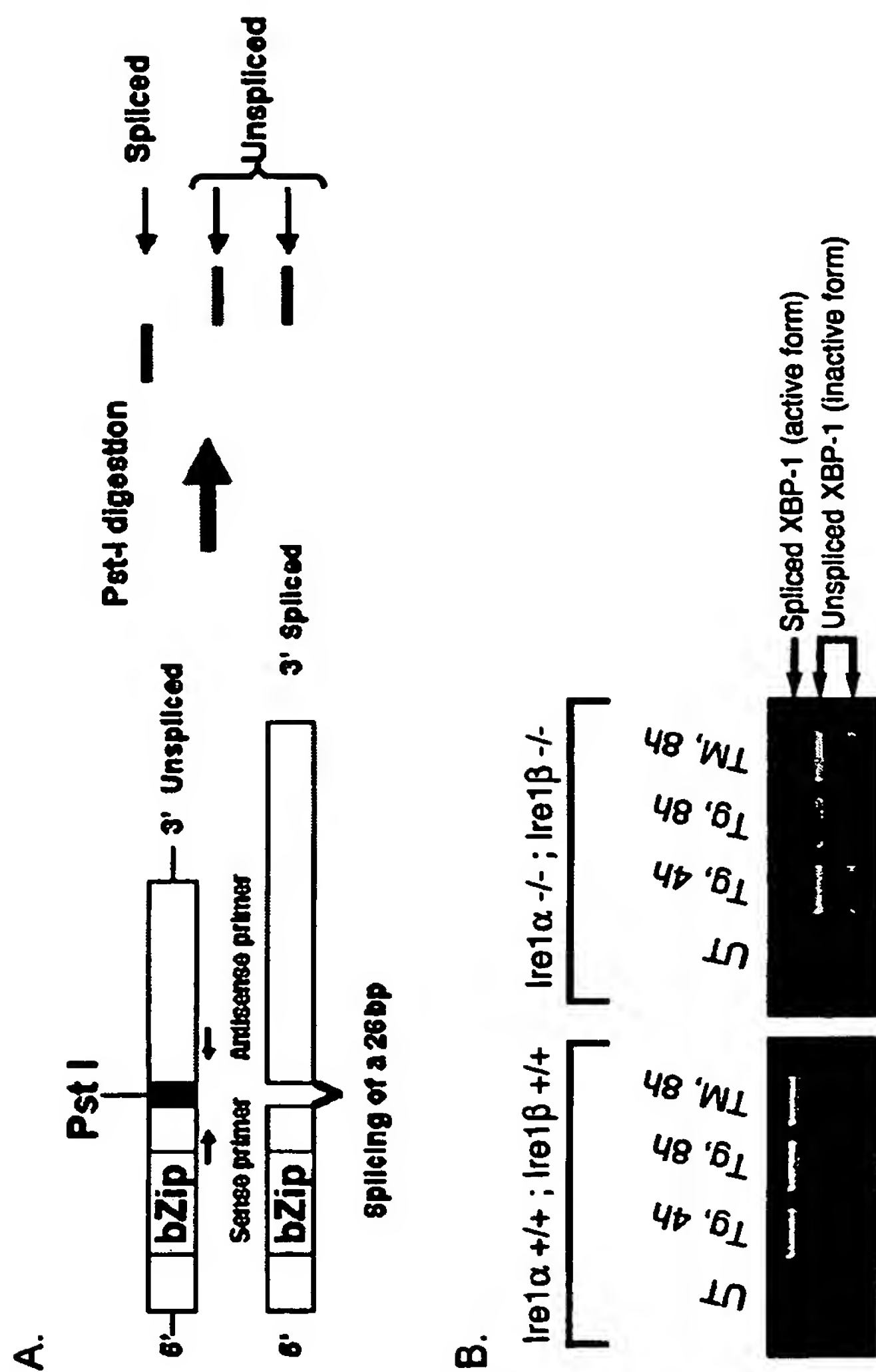


Fig. 1



Title: Methods and Reagents to Quantify Endoplasmic
Reticulum Stress (ER Stress)
Inventor: Fumihik Urano
Att may Docket No.: UMY-088-1
U.S. Serial No.: Not Yet Assigned
Sheet 3 of 4

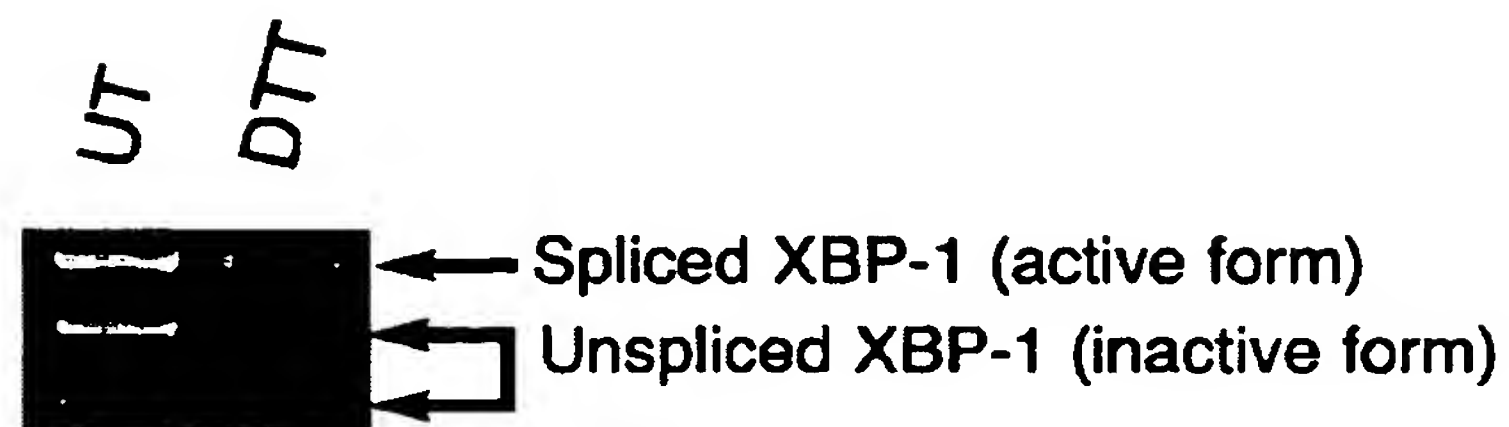


Fig. 3

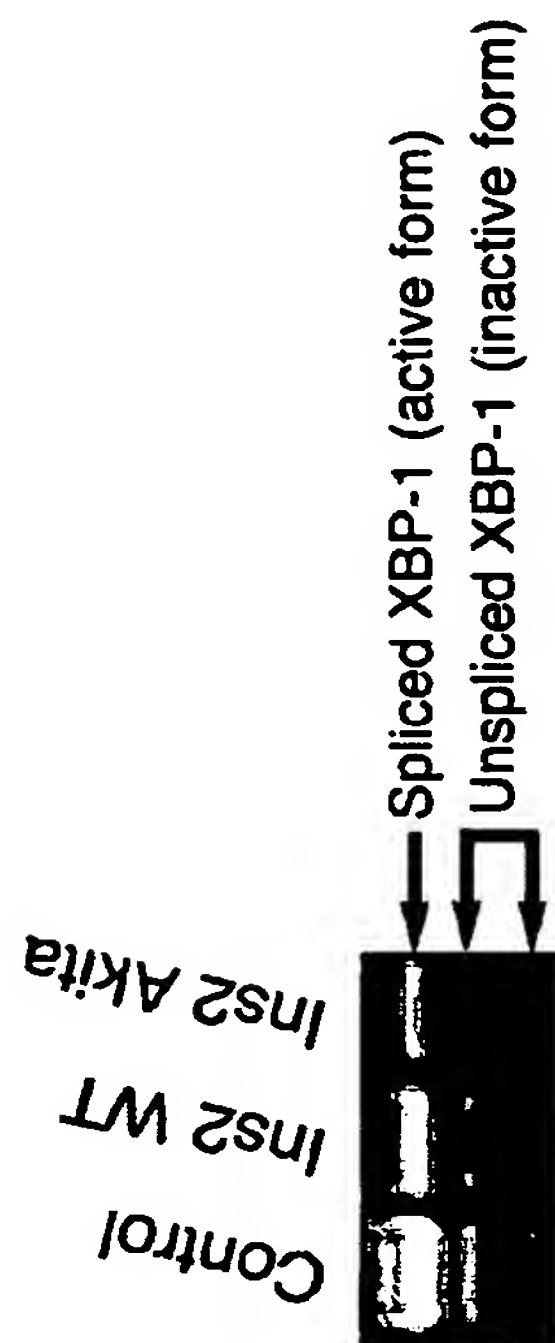


Fig. 4

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